

# Vasopressin Prolongs Behavioral and Cardiac Responses to Mild Stress in Young But Not in Aged Rats

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BUWALDA, B., C. NYAKAS, J. M. KOOLHAAS, P. G. M. LUITEN AND B. BOHUS. *Vasopressin prolongs behavioral and cardiac responses to mild stress in young but not in aged rats.* *PHYSIOL BEHAV* 52(6) 1127–1131, 1992.—In young male Wistar rats sudden silence superimposed on low intensity background noise evokes a relative decrease in heart rate. This bradycardia is accompanied by immobility behavior. In the present study, involving young (3 month), late-adult (14 month), aged (20 month), and senescent (25 month) rats the magnitude of the stress-induced bradycardia shows an age-related reduction while the behavioral immobility response remained unchanged during the process of aging. Arginine-8-vasopressin (AVP, 6 µg/kg SC) administered 60 min prior to the experiment led to a prolonged behavioral and cardiac stress response in young and late-adult rats, but not in aged and senescent animals. The peripheral and central mechanisms possibly involved in the failure of systemically applied AVP to improve bradycardiac stress responses in aged rats are discussed.

Aging    Immobility behavior    Autonomic responses    Bradycardia    Stress    Vasopressin

THE neurohypophyseal peptide arginine-8-vasopressin (AVP) modifies various forms of learned behavior, apart from its well-known classical endocrinological actions. Several studies showed that both systemically and centrally administered AVP prolongs extinction and improves consolidation and retrieval processes in avoidance learning tasks (10,19,23,25,37). A number of studies have focussed on the effects of AVP on physiological responses related to emotion and behavioral adaptation (4,5,20). Experiments in freely behaving adult rats in various stressful situations showed that AVP serves as an important modulator of a bradycardiac response to an emotional stressor (4,20). Because this stress-induced bradycardia appears to be vagally mediated (8), these experiments suggest that AVP is involved in a neural network that serves a parasympathetically regulated response to stress.

Previous research in this laboratory focussed on the age-related changes in the autonomic responses due to stressors inducing behavioral immobility (30). During this passive way of coping, young male Wistar rats reacted predominantly with a vagally mediated cardioinhibitory response. While the behavioral responsiveness to stress remained intact, the initial bradycardiac response appeared to be diminished in aged rats (30). Therefore, an age-related attenuation of parasympathetic control of cardiac functioning during emotional stress situations was suggested (30). Peripherally applied AVP appeared to reinstate the bradycardiac response to a conditioned stress of fear of inescapable footshock

in 14-month-old male Wistar rats (5,29). These results led to the hypothesis that AVP-dependent mechanisms are involved in the age-related reduction of stress-induced parasympathetic responsiveness. Because the bradycardiac response to conditioned aversive stimuli is thought to reflect the attentional demands of the experimental situation (31), one would expect a comparable action of AVP in nonaversive conditions that result in vagal activation due to acute orientation/attention behavior. Sudden silence superimposed on low intensity background noise is eliciting bradycardia and immediate behavioral arrest in young rats (18). In a previous study, AVP was demonstrated to enhance behavioral and cardiac responses to this sudden silence stress in young male rats, whereas it failed to improve or even worsened such responses in 24-month-old rats (7).

The aim of this paper was to analyze the modulating properties of AVP on behavioral and cardiac responses to the mild stress of sudden silence not only in young and old rats, but also in rats of intermediate ages to study at which moment a hypo-sensitivity to vasopressinergic modulation of stress-related behavior and cardiac control develops during the process of aging.

## METHOD

### *Animals and Housing*

Male Wistar rats of four different ages were used. The animals were 4, 14, 20, and 25 months old and originated from the

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Winkelman substrain (kindly donated by Troponwerke, Cologne, Germany). They were housed six to a cage (40 × 60 × 15 cm), with food and water ad lib, in a temperature-controlled environment of  $21 \pm 2^\circ\text{C}$ ; the lights were on from 0730 to 1930 h. All experiments were performed between 0900 to 1300 h.

### Surgery

In order to record the electrocardiogram (ECG), transcutaneous stainless steel electrodes made of standard paperclips were implanted under light ether anesthesia. One electrode was placed between the scapulae and the other in the midback region, according to the method described previously (3). At least 3 days were allowed for recovery before the start of the experiment.

### Recording and Analysis of the ECG

The ECG of freely moving rats was monitored telemetrically by means of a miniature FM transmitter (model SNR 102F, Dynamic Electronics Ltd., London, England) as described before (3). The transmitter was attached to a velcro strap secured around the chest of the rat and connected to the transcutaneous electrodes. The transmitted signals were received on a commercial FM receiver, amplified (Narco Bio-System Inc. Mod. FM-1100-7), and stored on tape by a commercial tape recorder. During recording and analysis, the quality of the ECG signal was continuously monitored on an oscilloscope.

Recorded ECG samples were played back through a cardiometer pulse generator (Schmitt-trigger) that generated a square wave pulse at each R wave. The interbeat interval (IBI), i.e., the time elapsed between onset of the two consecutive pulses, was measured using a personal computer (Olivetti M24). The mean IBIs were computed for periods of 55 s. IBIs shorter than 100 and longer than 220 ms were discarded because these were likely to be due to artifacts.

### Procedure

The behavioral and cardiac responses to a sudden drop in background noise were measured in a rectangular clear Plexiglas cage (85 × 60 × 60 cm), designated as an open field in this paper. This open field with a wood shavings covered floor was located in an acoustically isolated experimental room where a noise generator produced a background noise that was maintained at a constant level (65 dB, 2–8 kHz). Upon entering, a velcro strap holding a miniature FM-transmitter for the ECG recordings was fixed around the chest of the rat. The animal was placed in the open field for 5 min on day 1. On day 2, the test day, the animals were exposed again to the open field for 5 min. After 2 min the background noise was switched off, leaving the animal in almost total silence for the remaining 3 min. Heart rate and behavior were recorded for three periods (P) of 60 s. Second-min recordings were made during the 60 s immediately prior to switching off the background noise (P1). Third-min recordings (P2) during the 60 s immediately after switching off the noise (P2) and fifth-min recordings (P3) were regarded, respectively, as response and recovery measurements. As a behavioral measure, the time spent immobile was determined by an observer during the three periods. Immobility was defined as almost motionless scanning of the environment with only minor head movements.

### Treatment

Arginine-vasopressin (AVP) was dissolved in saline and injected subcutaneously (SC) in a dosage of 6  $\mu\text{g/kg/ml}$  60 min prior to the exposure to the open field. Saline injections served as vehicle. The selection of the single dose of the peptide was

based on the results of the previous studies of young and aged rats' cardiac response to emotional stress (29) and on several other studies describing behavioral and physiological effects of peripherally applied AVP (20–22,24,25). EEG recordings (13) further indicated a similarity between SC administered AVP in a dose of 6  $\mu\text{g/kg}$  and a behaviorally relevant intracerebroventricular administered dose of 1 ng. The rats were injected in a crossover design with saline or AVP, each animal serving as its own control. A 7-day wash-out period was allowed between injections to minimize interaction of treatments.

### Statistics

The results are presented as means  $\pm$  SEM in two measures; absolute values are presented in the figures and response (P2–P1) and recovery (P2–P3) values in the table. The absolute cardiac data were analyzed using a multivariate ANOVA (two-way) utilizing one between-subjects factor (group) and one repeated measures within-subjects factor (periods), a two-tailed Student's *t*-test and a paired *t*-test. Response and recovery values were analyzed using a one-way ANOVA utilizing the factor age. Behavioral data were evaluated for significance using the Kruskal–Wallis ANOVA and the Wilcoxon matched-pairs ranked-signs test. A probability level of  $p < 0.05$  was taken as statistical significance for all tests.

## RESULTS

### Vasopressinergic Modulation of Cardiac Stress Responses

Figure 1 shows the heart rate values, expressed as IBI, of the animals in the open field and the cardiac response to stress of sudden silence. ANOVA testing revealed no effect of age on presilence, i.e., prestress values during P1. The magnitude of response as well as the recovery values after sudden cessation of background noise are presented in Table 1. Multivariate ANOVA with repeated measures for the three periods showed no significant age effect in vehicle-treated animals, but a highly significant

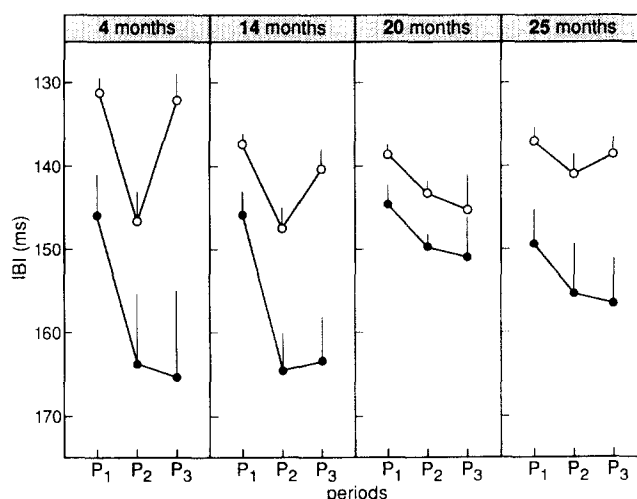


FIG. 1. Heart rate expressed as interbeat interval (IBI) during a 5-min exposure to an open field before and after sudden reduction of a non-aversive background mixed noise, measured in 4 ( $n = 9$ ), 14 ( $n = 9$ ), 20 ( $n = 8$ ) and 25 ( $n = 11$ )-month-old male Wistar rats. One-min recordings were made during the second min of exposure to the open field with the noise on (P1); during the third min immediately after the noise was switched off (P2), and during the fifth min (P3). IBIs are presented after subcutaneous administration of saline (open circles) and AVP (closed circles). Means  $\pm$  SE are shown.

interaction between age and periods,  $F(2, 64) = 4.7, p = 0.0007$ . Although all vehicle-treated age groups showed a significant cardioinhibitory response, i.e., an increase in IBI, to switching off the noise, the magnitude of this response (see Table 1) showed an age-related decrease,  $F(3, 32) = 6.27, p = 0.002$ . Recovery, measured as the difference in IBI between P3 and P2 was also reduced in aging,  $F(3, 32) = 6.73, p = 0.002$ .

Administration of AVP caused a decrease in heart rate already before the reduction of background noise, i.e., P1, in the open field in all age groups, expressed in a significant treatment effect,  $F(1, 65) = 21.7, p < 0.001$ . There was no significant interaction between age and treatment on prestress values. Multivariate ANOVA for the factors age by treatment by periods also showed no significance. The interaction between age and periods as shown after vehicle treatment disappeared after AVP administration, suggesting an effect of the treatment throughout the periods. This is caused by the absence of recovery during P3 of the cardiac stress response in 4- and 14-month-old rats receiving AVP. A significant interaction between treatment and periods was observed in 4-,  $F(2, 32) = 5.88, p = 0.007$ , and 14-month-old rats,  $F(2, 32) = 3.17, p = 0.05$ . Twenty- and 25-month-old rats failed to show this interaction.

#### Vasopressinergic Modulation of Behavioral Stress Responses

ANOVA testing revealed no significant age or treatment effect on prestress immobility values. Neither was an interaction between age and treatment present during P1. Figure 2 shows that all vehicle-treated animals responded to sudden cessation of background noise with an increase in immobility behavior (see also Table 1). ANOVA (Kruskal-Wallis) indicated that neither the magnitude of the response nor the recovery after the sudden stimulus change were affected by age. Only 20-month-old animals failed to show a significant recovery of immobility behavior after stress.

After AVP administration there was an effect of age on immobility responses,  $H(3, 37) = 14.95, p = 0.002$ . Post hoc testing indicated that in 14-month-old rats AVP increased the immobility response ( $p < 0.05$ ), whereas in 25-month-old rats the behavioral stress response was reduced ( $p < 0.01$ ). A two-way ANOVA showed a significant interaction between the factors

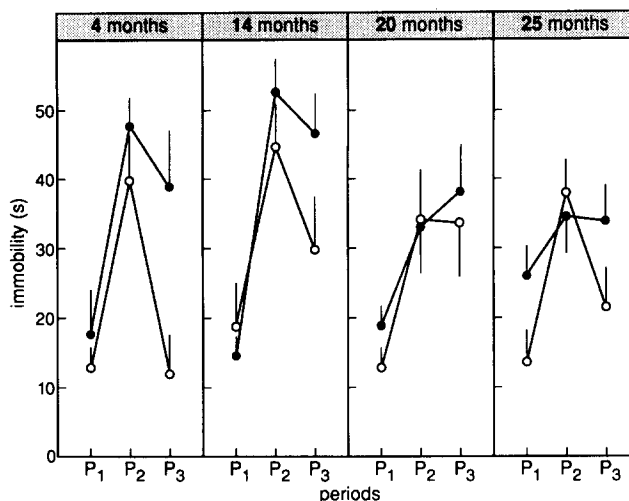


FIG. 2. Behavioral immobility in the open field before (P1) and after sudden silence (P2 and P3) in 4-, 14-, 20-, and 25-month-old rats. For further information see Fig. 1.

age and treatment on immobility responses,  $F(3, 65) = 2.8, p = 0.04$ .

#### DISCUSSION

The present findings indicate that between the age of 14 and 20 months, male Wistar rats develop a hyposensitivity to AVP in modifying the behavioral and cardiac response to mild unexpected stress.

AVP caused a reduction in heart rate in all age groups already before the sudden reduction of background noise. This may be the result of a baroreceptor reflex-induced vagal activation. That this alternative is unlikely is suggested by our former study (29). Sixty min after peripheral administration of 10  $\mu\text{g/kg}$  AVP a moderate bradycardia was found in rest conditions, together with a slightly decreased blood pressure. Lebrun et al. (24) also showed that the systolic pressor response to 6  $\mu\text{g/kg}$  AVP dis-

TABLE 1  
RESPONSE VALUES TO (P2-P1) AND RECOVERY AFTER (P2-P3) THE SUDDEN CESSATION OF BACKGROUND NOISE AFTER SALINE OR AVP APPLICATION

| Rat Age   | Response to Sudden Silence |                               | Recovery After Sudden Silence |                               |
|-----------|----------------------------|-------------------------------|-------------------------------|-------------------------------|
|           | $\Delta\text{IBI (ms)}$    | $\Delta\text{Immobility (s)}$ | $\Delta\text{IBI (ms)}$       | $\Delta\text{Immobility (s)}$ |
| 4 Months  |                            |                               |                               |                               |
| SAL       | $15.4 \pm 3.7$             | $26.2 \pm 3.6$                | $14.1 \pm 3.6$                | $28.0 \pm 6.3$                |
| AVP       | $18.1 \pm 4.2$             | $30.3 \pm 5.4$                | $1.7 \pm 3.7^*$               | $8.7 \pm 6.0^\dagger$         |
| 14 Months |                            |                               |                               |                               |
| SAL       | $10.0 \pm 1.7$             | $24.9 \pm 6.1$                | $7.2 \pm 1.6$                 | $14.7 \pm 7.0$                |
| AVP       | $18.8 \pm 4.7$             | $37.4 \pm 4.4^\dagger$        | $0.9 \pm 5.2$                 | $6.2 \pm 5.8$                 |
| 20 Months |                            |                               |                               |                               |
| SAL       | $4.8 \pm 1.8$              | $19.0 \pm 6.2$                | $-2.1 \pm 4.0$                | $-0.1 \pm 14.0$               |
| AVP       | $5.2 \pm 2.7$              | $13.9 \pm 5.4$                | $-0.7 \pm 3.8$                | $-5.0 \pm 6.2$                |
| 25 Months |                            |                               |                               |                               |
| SAL       | $3.5 \pm 0.9$              | $23.7 \pm 3.6$                | $2.3 \pm 1.1$                 | $16.4 \pm 4.3$                |
| AVP       | $6.0 \pm 3.0$              | $8.7 \pm 5.0^*$               | $-1.2 \pm 2.0$                | $0.7 \pm 4.4^*$               |

\*† Within the age group the response and recovery values after AVP administration were compared with the animals' own vehicle control value by means of a paired *t*-test (cardiac data) or a Wilcoxon matched pairs-ranked signs test (behavioral data), \* $p < 0.01$ , † $p < 0.05$ .

appears within 60 min after administration. Therefore, the reduction of heart rate may be caused by a direct action of vasopressin on the heart (14,35) through effects of the peptide on coronary blood flow and oxygen availability within the myocardium (2,27).

In addition to the general reduction in heart rate, AVP extended the bradycardiac stress response in 4- and 14-month-old animals. These data support the finding of an effect of AVP on acute cardiac stress responses in young rats as described previously (20).

Administration of AVP enhanced the magnitude of the behavioral immobility response to stress in 14-month-old animals, whereas it decreased this response in 25-month-old rats. This differential age-related effect of AVP on the behavioral immobility responses is reflected in the significant interaction between age and treatment. Peptide treatment further prolonged the duration of immobility behavior in 4-month-old animals.

Both centrally (10) and peripherally (25) located mechanisms are hypothesized to be involved in vasopressinergic modulation of stress-related autonomic and behavioral responses. The extension of the bradycardiac stress responses may reflect a sensitization of baroreflex control of circulation (16). No data are available, however, on age-related changes in the vasopressinergic modulation of these reflexes. In numerous studies changes of vasopressinergic innervation patterns in the brain of aged rodents was shown [for review see (36)]. Age-related changes in vasopressin cells in the suprachiasmatic nucleus were reported (34). Dorsa and Bottemiller (12) found decreased AVP concentrations in a number of intra- and extrahypothalamic areas in aged rats, e.g., in septum, the vascular organ of the lamina terminalis, and the locus coeruleus. A study of Fliers et al. (15) revealed that age-related reduction in vasopressinergic innervation occurs in many areas of the brain controlling behavioral and autonomic responses. Only a few studies have been devoted to the effects of exogenous AVP in behavioral tasks in aged animals. While the present study addresses the effects of AVP on attentional processes, most of these studies involve age-related effects of AVP on memory measures. In late-adult rats (13–14-months old) AVP enhanced cardiac and behavioral stress responses (5) and improved memory function (37). Cooper et al. (9) showed that AVP facilitated the conditioned taste aversion in 19- and 24-month-old rats.

While the present findings in the younger groups confirm the reported effects of AVP on behavioral and autonomic performance (5,20,25), the failure of the peptide to elicit similar effects in aged rats indicate a decreased sensitivity to AVP in these old

animals. A cause of the vasopressinergic hyposensitivity in aged rats may be a decreased sensitivity or number of vasopressinergic receptors during aging. No information to our knowledge is available yet about age-related alterations in the properties of vasopressinergic receptors in the brain. Peripherally, however, a strong decrease in vasopressin binding was found in the kidney (33) and the liver (26) of aged rats. Administration of AVP failed to correct the impaired urine concentration in aged rats (1), indicating the decreased sensitivity of the aged kidney to the antidiuretic effect of AVP. However, vascular smooth muscles of old rats show an increased sensitivity to AVP (17). Accordingly, there are probably organ-specific differences in the sensitivity to vasopressin during aging, i.e., age does not affect all vasopressinergic receptors uniformly. A third possible mechanism of the reduced effect of AVP in aged rats might be related to age-dependent changes in receptor-stimulated second-messenger activation (38). There is extensive evidence that central and peripheral catecholaminergic systems interact with neuropeptides such as AVP in the modulation of behavior (6,23,32). Because peripherally applied d-amphetamine enhances the bradycardiac stress response in aged rats (28), it is also possible that AVP indirectly modulates the cardiodeceleratory response to emotional stress through activation of aminergic systems in the brain. The failure of AVP to restore the bradycardia in old animals, therefore, can be related to the reported age-related reduced activation of central catecholaminergic systems (39).

Whether the effects of systemically applied AVP in affecting cognitive processes are peripherally or centrally mediated is still an issue of major discussion (11,22). Whereas De Wied and colleagues (10) suggested a direct central action of vasopressin, Le Moal et al. (25) hypothesized that hemodynamic responses shortly after the peptide injection were causing the behavioral effects of AVP. Koob et al. (21) suggested that systemically and centrally administered AVP can influence behavior in a homologous manner but by different mechanisms of action. Our data fit with the decreased peripheral sensitivity, but a central direct action cannot be excluded.

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